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TRADEMARK OFFICE

TRANSMITTAL LETTER (General - Patent Pending)

Docket No. RU-0075

In Re Application Of: Anderson and Montelione

Serial No.	Filing Date	Examiner	Group Art Unit
09/181,601	October 29, 1998	J. Fredman	1634

Title: **LINKING GENE SEQUENCE TO GENE FUNCTION BY THREE DIMENSIONAL (3D) PROTEIN STRUCTURE DETERMINATION**

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Transmitted herewith is:

Appeal Brief (Small Entity)
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Dated: **September 16, 2003**

Signature

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1) General Transmittal Letter (in triplicate);

2) Appeal Brief (in triplicate);

3) Return Post Card; and

4) Credit Card Payment Form for $160.00.

Jane Massey Licata
JANE MASSEY LICATA

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No.: **RU-0075**

Inventors: **Anderson and Montelione**

Serial No.: **09/181,601**

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Title: **Linking Gene Structure to Gene Function by Three Dimensional (3D) Protein Structure Determination**

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Dear Sir:

APPEAL BRIEF

Table of Contents

I. Real Party of Interest

The real party of interest is Rutgers, The State University of New Jersey, assignee of all rights, title and interest in the instant application.

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Claims 2, 15, 16 and 17 are canceled.

Claims 1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 and 14 are pending.

Claims 1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 and 14 are rejected.

A copy of pending claims 1 and 3 through 14 is attached hereto as Appendix 1.

It was also noted during preparation of this appeal brief that claim 3 has an inadvertent error in its reference to a canceled claim, claim 2. Accordingly, claim 3 has been amended in this brief to refer to the proper independent claim, claim 1, and can be found in Appendix 2.

IV. Status of Amendments

All amendments to the claims have been entered upon this appeal.

V. Summary of the Invention

The claimed invention is a method for elucidating the function of proteins and protein domains by generating and examining their three dimensional structures, and more specifically by the use of bioinformatics, molecular biology and nuclear magnetic resonance spectroscopy to enable the rapid and automated determination of functions, as a means for genome analysis. In this method, a target polynucleotide which encodes a protein of unknown function is first parsed into at least one putative polypeptide domain. This parsing step is discussed at page 5, lines 23-25, page 7, lines 3-7 and lines 20-23, and page 10, lines 23-30 of the specification as filed. The second step of the instant method involves identifying a putative polypeptide domain consisting of 50 to 300 amino acids that properly folds into a stable polypeptide domain consisting of 50 to 300 amino acids. This identification step involving a domain of the claimed size range is taught at page 11, lines 29-34 of the specification as filed. In the third step of the claimed method, the three dimensional structure of the stable polypeptide domain is then determined. Methods for determining the three dimensional structure of the stable

polypeptide domain are taught at pages 3-5 and 9-10. The next step involves comparing the determined three dimensional structure of the stable polypeptide domain to known three dimensional structures in a protein data bank in order to identify known structures within the protein data bank that may be homologous to the determined three dimensional structure. This step in the method is discussed at page 24, lines 25-35. The final step in the claimed method involves correlating a biochemical function corresponding to the identified homologous structure to a biochemical function for the stable polypeptide domain. This part of the method is described at page 25, lines 30-35 and page 26, lines 1-35. Further, the method of the present invention is shown graphically as a flow chart in Figure 1.

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The issues on appeal are:

1) whether claims 1, 3-6 and 11-14 are unpatentable under 35 U.S.C. § 103(a) as being obvious over Wallace et al. (1996), in view of Holm et al. (1995), and further in view of Farber et al. (1992);

2) whether claims 1, 5-9 and 11-14 are unpatentable under 35 U.S.C. § 103(a) as being obvious over Wallace et al. (1996), in view of Holm et al. (1995), and further in view of Friedrichs et al. (1994);

3) whether claims 1 and 5-14 are unpatentable under 35 U.S.C. § 103(a) as being obvious over Wallace et al. (1996), in view of Holm et al. (1995), and further in view of Friedrichs et al. (1994) and Bagby et al. (1997); and

4) whether claims 1, 3-9 and 11-14 are unpatentable under 35 U.S.C. § 103(a) as being obvious over Wallace et al. (1996), in view of Holm et al. (1995), and further in view of Farber et al. (1992) and Friedrichs et al. (1994).

VII. Grouping of Claims

Claims 1 and 3-14 stand or fall together on the issue of obviousness under 35 U.S.C. § 103(a) over any combination of the cited art.

VIII. Arguments

A. Rejection of Claims Under 35 U.S.C. 103(a)

There are four pending rejections under 35 U.S.C. 103(a) and each rejection relies on the same primary reference. The four separate rejections then involve four different combinations of secondary references.

The Examiner has rejected claims 1, 2-6 and 11-14 under 35 U.S.C. § 103(a) as being unpatentable over Wallace et al. (1996), in view of Holm et al. (1995), and further in view of Farber et al. (1992). The Examiner suggests that Wallace et al. (1996) disclose

methods for identification of domains within proteins while Holm et al. (1995) disclose determination of 3D structure by crystallography or NMR followed by database analysis using the DALI method. The Examiner suggests that Holm et al. (1995) disclose a comparison between urease and adenosine deaminase in which the complete 3D structure of the 352 amino acid adenosine deaminase protein is compared to the larger urease and where a comparison was performed for Adenovirus type 5 knob domain of 195 amino acids. The Examiner acknowledges that the combination of these two references does not teach or suggest the step of parsing a target polynucleotide into at least one putative polypeptide domain. However, the Examiner suggests that Farber et al. (1992) discloses a method for discriminating open reading frames in DNA, and that it would have been obvious for one of ordinary skill to combine these three references in order to maximize the useable databases to identify homologous proteins and thereby determine function of unknown proteins.

Although the Examiner was silent as to the status of this rejection in the Advisory Action dated March 3, 2003, this rejection was maintained in the last Office Action dated September 16, 2002. The Examiner rejected claims 1, 5-9 and 11-14 under 35 U.S.C. § 103(a) as being unpatentable over Wallace et al. (1996), in view of Holm et al. (1995), and further in view of Friedrichs et al. (1994). The Examiner suggests that Wallace et al. (1996)

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Again, although the Examiner was silent as to the status of this rejection in the Advisory Action dated March 3, 2003, this rejection was maintained in the last Office Action dated September 16, 2002. The Examiner rejected claims 1 and 5-14 under 35 U.S.C. § 103(a) as being unpatentable over Wallace et al. (1996), in view of Holm et al. (1995), and further in view of Friedrichs et al. (1994) and Bagby et al. (1997). The Examiner suggests that Wallace et al. (1996) disclose methods for identification of

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Finally, with respect the fourth remaining rejection under 35 U.S.C. 103(a), although the Examiner was silent as to the status of this rejection in the Advisory Action dated March 3, 2003, this rejection was maintained in the last Office Action dated September 16, 2002. The Examiner has rejected claims 1-9 and 11-14 under 35

U.S.C. § 103(a) as being unpatentable over Wallace et al. (1996), in view of Holm et al. (1995), and further in view of Farber et al. (1992) and Friedrichs et al. (1994). The Examiner suggests that Wallace et al. (1996) disclose methods for identification of domains within proteins while Holm et al. (1995) disclose determination of 3D structure by crystallography or NMR followed by database analysis using the DALI method. The Examiner suggests that Holm et al. (1995) disclose a comparison between urease and adenosine deaminase in which the complete 3D structure of the 352 amino acid adenosine deaminase protein is compared to the larger urease and where a comparison was performed for Adenovirus type 5 knob domain of 195 amino acids. The Examiner acknowledges that the combination of these two references does not teach or suggest the step of parsing a target polynucleotide into at least one putative polypeptide domain. However, the Examiner suggests that Farber et al. (1992) discloses a method for discriminating open reading frames in DNA, and that it would have been obvious for one of ordinary skill to combine these three references in order to maximize the useable databases to identify homologous proteins and thereby determine function of unknown proteins. The Examiner suggests that Friedrichs et al. (1994) teaches the use of NMR techniques.

Appellant respectfully disagrees with the Examiner's suggestions regarding each of the four rejections under 35 U.S.C. 103(a) that are on appeal.

1. Summary of the Teachings of Each Reference Cited Under 35 U.S.C. 103(a)

Wallace et al. (1996) teach derivation of 3-dimensional coordinate templates that have been derived from known 3-dimensional protein structures which are provided in a database. The paper then teaches determination of biochemical function based on the existence of the known 3-dimensional structures. The critical step in the method is the identification of a triad of amino acids, Ser-His-Asp, that occur in a 3-dimensional configuration to form an active site of a domain. No other size of a putative domain is taught or suggested by this reference. In fact, it is this 3 amino acid sequence that is then used to guide identification of the functions of the unknown proteins as the authors report that this triad is what is critical for differentiating catalytic from non-catalytic proteins among the serine proteases and lipases. As a result, this paper would teach away from any suggestion that a domain of 50 to 300 amino acids, such as claimed in the instant invention, would be useful for comparison to protein data bases and then identifying

proteins. Further, as the authors state at page 1002, last paragraph in the first column, their method had specific steps that involved using a data set of serine proteases and lipases to automatically compute a highly specific 3D template for the Ser-His-Asp catalytic triad in the set of proteins. As they also state at page 1002, top of the second column, their method differed from other previous methods in that a simple template specific to the Ser-His-Asp catalytic triad is derived. Then, the next step was to search for similar triads in other proteins to see how often they occurred outside of the serine proteases and lipases. Thus, the teaching of this paper is limited to teaching a skilled artisan that databases may be searched using a specific 3D template, the triad, and then used to identify a potential biological function of an unknown protein. This paper also fails to teach either the prestep of the instant claims that involves parsing a database to identify a protein coding region, as well as failing to provide any motivation to predict exon boundaries in a nucleotide sequence to identify protein domains.

Holm et al. (1995) is a commentary article wherein the DALI method is disclosed as being useful for the study of protein structure. In this method, structural relatedness is measured in terms of similarities of intramolecular distance matrices. The importance of this new automated method is

discussed. Although the Examiner suggests that this paper teaches a comparison that was performed on the Adenovirus type 5 knob domain which is a 195 amino acid comparison, nowhere does this paper teach or suggest that there was specifically such a size comparison. In fact, the table referred to by the Examiner (table 1) contains no such useful size information. Accordingly, contrary to the Examiner's suggestion, this paper does not teach a method as claimed which recites identifying a putative polypeptide domain consisting of 50 to 300 amino acids. This paper also fails to teach a method which has any specific steps at all, including a prestep of parsing a database to identify a protein domain. It also does not suggest performing a structural analysis of a protein of unknown function.

Farber et al. (1992) disclose a neural network and information theory for determination of coding regions of DNA sequence. This paper, from a different art area (not protein biochemistry as is the are of one of skill in the instant invention), fails to teach or suggest a method such as the claimed invention. Nowhere is there any teaching or suggestion of the identification of protein or polypeptide domains of 50 to 300 amino acids. Moreover, being in a different art area, this paper would not be one that would be

used by one of skill in protein biochemistry as motivation to produce the method of the instant invention.

Friedrichs et al. (1992) is a review type paper that teaches an automated NMR procedure. This paper fails to teach or suggest a method with specific steps such as is claimed in the instant invention. In particular, there is no teaching of first parsing a target polypeptide that encodes a protein of unknown function into putative polypeptide domains, followed by the identification of protein or polypeptide domains of 50 to 300 amino acids, and then, after use of NMR techniques for protein structure determination, correlating structure with biochemical functions.

Bagby et al. (1997) teach methods for preparing samples for NMR analysis, specifically a button test method. This method is used by the authors to screen solution conditions to determine conditions under which a protein is soluble at high concentrations that are used for NMR spectroscopy. This paper fails to teach or suggest a method with specific steps such as is claimed in the instant invention. In particular, there is no teaching of first parsing a target polypeptide that encodes a protein of unknown function into putative polypeptide domains, followed by the identification of protein or polypeptide domains of 50 to 300 amino acids, and then, after

use of NMR techniques for protein structure determination, correlating structure with biochemical functions.

Therefore, Appellants respectfully disagree with the Examiner's suggestion that the cited combination of prior art references establishes a *prima case* of obviousness.

2. If an Independent Claim is Nonobvious Then a Claim Depending Therefrom is Nonobvious (MPEP 2143.03)

At the outset, Appellants will address the four rejections under 35 U.S.C. as a group since in each case the only independent claim, claim 1, has been included in the rejections. Appellants respectfully point out that under MPEP 2143.03, "If an independent claim is nonobvious under 35 U.S.C. 103, then any claim depending therefrom is nonobvious (In re Fine, 837 F.2d 1071, 5 USPQ2d 1596, Fed. Cir. 1988)."

3. Three Basic Criteria of *prima facie* Obviousness

In accordance with MPEP § 2143, to establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the reference itself or in the knowledge generally available to one of ordinary skill in the art, to modify the reference. Second, there must be a reasonable expectation of success. Finally, the prior art must teach or suggest all of the claim

limitations. The prior art combinations cited under 35 U.S.C. 103 by the Examiner fail to teach or suggest all of the limitations of the claims, teach toward other limitations, and fail to provide either the motivation to modify the reference or the expectation of success.

4. The Cited Art Fails to Teach the Limitations of the Claims

None of the suggested combinations of prior art teach or suggest two of the limitations in the claims, namely the use of putative polypeptide domains of 50 to 300 amino acids and the pre-step of parsing the target polypeptide, one that encodes a protein of unknown function, into at least one putative polypeptide domain. The primary reference cited by the Examiner, Wallace et al. (1996), fails to teach or suggest either of these limitations as discussed above. In fact, Wallace et al. (1996) teaches the critical nature of a triad. In considering the secondary references cited, the fact that one comparison in the paper by Holm et al. (1995) happened to be 195 amino acids is not the same as teaching or suggesting that size is an important factor as is claimed in the instant invention. Both the MPEP and case law are quite clear. To establish *prima facie* obviousness of a claimed invention, all the limitations must be taught or suggested by the prior art.

In re Royka, 490 F.2d 981, 180 USPQ 580 (CCPA 1974) and MPEP 2143.03. Since none of the prior art references, either alone or when combined, teach or suggest the limitations of first parsing a target polypeptide, one that encodes a protein of unknown function, into at least one putative polypeptide domain and then identifying a putative polypeptide domain that properly folds into a stable polypeptide domain of 50 to 300 amino acids, the cited combination of art cannot render the instant invention obvious as set forth in claim 1 or dependent claims therefrom because there is no explicit teaching of these limitations.

5. The Combined References Fail to Provide Motivation

The cited combinations of references also fail to establish a *prima facie* case of obviousness because the motivation to combine these references is lacking. The teaching or suggestion must be found in the prior art and not based on applicant's disclosure. *In re Vacek*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). The mere fact that the cited references can be combined or modified does not render the resultant combination obvious unless the prior art suggests the desirability of the combination. MPEP 2143.01. *In re Mills*, 916 F.2d 680, 16 USPQ2d 1430 (Fed. Cir. 1990). It is only the hindsight vision afforded by the claimed invention

could provide motivation to consider Wallace et al., Holm et al., and Farber et al. Wallace et al. and Holm et al. are protein biochemistry articles directed at the analysis of protein structure and function. Farber et al. is from the art area of information theory. Contrary to the Examiner's suggestion, it would not have been obvious to the ordinary protein biochemist after reading these disclosures to take the additional step of predicting exon boundaries in a polynucleotide sequence to identify protein domains.

Further, since the reference of Wallace et al. teaches the importance of their triad domain, one of skill in the art would not be motivated to look for some other size limitation to apply to a method such as the claimed method.

Therefore, solely on the basis of the requirements for establishing a *prima facie* case of obviousness (MPEP 2143), any of the combinations of the cited references fail to make claim 1, and by dependency claims 3-14, obvious.

6. The Cited References Fail to Provide a Reasonable Expectation of Success

The primary reference cited in each of the four remaining 103 rejections, Wallace et al. (1996), is also important in that it would actually teach away from an expectation of success for the present method. As discussed in detail *supra*,

the method of Wallace et al. is based on the importance of the identification of the triad domain. It is only with use of this domain as a screening tool that the authors report any success at correlating structure of a protein with a function. The present method, however, is not based on this triad, and in fact is based on a very different type of domain. Accordingly, this primary reference would lead one of skill to not expect success using the current method which is not limited to use of the triad disclosed by Wallace et al.

Therefore, solely on the basis of the requirements for establishing a *prima facie* case of obviousness (MPEP 2143), these combined references, in any cited combination, fail to make claims 1 and 3-14 obvious.

IX. Conclusion

The references cited by the Examiner clearly do not provide the requisite teaching or suggestion to render the claimed

invention obvious. Accordingly, it is respectfully requested that the rejections under 35 U.S.C. be withdrawn.

Respectfully submitted,

Jane Massey Licata

Jane Massey Licata
Registration No. 32,257

Date: **September 16, 2003**

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Appendix 1 - Pending Claims

Claim 1: A high-throughput method for determining the biochemical function of a protein or polypeptide domain of unknown three dimensional structure and function comprising:

(A) parsing a target polynucleotide into at least one putative polypeptide domain;

(B) identifying a putative polypeptide domain consisting of 50 to 300 amino acids that properly folds into a stable polypeptide domain consisting of 50 to 300 amino acids;

(C) determining three dimensional structure of the stable polypeptide domain;

(D) comparing the determined three dimensional structure of the stable polypeptide domain to known three-dimensional structures in a protein data bank, wherein said comparison identifies known structures within said protein data bank that are homologous to the determined three dimensional structure; and

(E) correlating a biochemical function corresponding to the identified homologous structure to a biochemical function for the stable polypeptide domain.

Claim 3: The method according to claim 2, wherein said parsing is performed by a first computer algorithm, wherein said first computer algorithm is selected from the group consisting of a computer algorithm capable of determining exon phase boundaries of a polynucleotide, and a computer algorithm capable of determining interdomain boundaries encoded in a polynucleotide.

Claim 4: The method of claim 3, further comprising a computer algorithm that compares the putative polypeptide domain sequence with known domain sequences stored within a database.

Claim 5: the method of claim 1, wherein said identification of the stable polypeptide domain having a defined three dimensional structure is performed by a set of activity-independent biophysical criteria that assesses the correctness of folding of the polypeptide domain said set of activity-independent biophysical criteria including at least one of the criteria selected from the group consisting of circular dichroism measurements, ^{1}H-NMR spectroscopy, amide hydrogen-deuterium time course exchange, and thermal denaturation.

Claim 6: The method of claim 1, wherein said determination of the three dimensional structure of the stable polypeptide domain is obtained from an NMR spectrometer spectra of said polypeptide domain.

Claim 7: The method of claim 6, wherein said NMR spectrometer spectra include one or more spectra selected from the group consisting of nuclear Overhauser effect spectroscopy (NOESY), pulsed-field gradient ^{15}N-heteronuclear single-quantum coherence spectroscopy (PFG-HSQC), pulsed-field gradient triple-resonance HCCNH^{13}C-^{13}C total correlation spectroscopy (PFG-HCCNH-TOCSY), pulsedfield gradient HCC(CO)NH^{13}C-^{13}CTOCSY (PFG-HCC(CO)NH-TOCSY), HCCH COSY, HCCNH-TOCSY, HNCO, CANH, CA(CO)NH, CBCNH, CBCA(CO)NH, H(CA)NH, and H(CA)(CO)NH.

Claim 8: The method of claim 6, wherein said NMR spectra is analyzed by a second computer algorithm that automatically assigns resonance assignments to the polypeptide sequence.

Claim 9: The method of claim 1, wherein said identification of said stable polypeptide domain comprises measuring a time course of amide hydrogen-deuterium exchange.

Claim 10: The method of claim 1, wherein prior to step (B), said polypeptide domain is optimally solubilized, said optimum solubilization comprising:

I) preparing an array of microdialysis buttons, wherein each of said microdialysis buttons contains at least 1 µl of an approximately 1 M solution of said stable polypeptide domain;

ii) dialyzing each member of said array of microdialysis buttons against a different dialysis buffer;

iii) analyzing each of said dialyzed microdialysis buttons to determine whether said stable polypeptide domain has remained soluble; and

iv) selecting the polypeptide domain having optimum solubility characteristics for NMR spectroscopy.

Claim 11: The method of claim 1, wherein said comparison of said determined three dimensional structure to said known three-dimensional structures in the protein data bank is performed by a third computer algorithm that is capable of determining 3D structure homology between said determined three dimensional structure and a member of said protein data bank.

Claim 12: The method according to claim 11, wherein said third computer algorithm is selected from the group consisting of DALI, CATH, AND VAST.

Claim 13: The method of claim 1, wherein said protein data bank is Protein Data Base ("PDB").

Claim 14: The method of claim 4, wherein said database contains domain sequence information of known and determined domain sequences.

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Inventors: Anderson and Montelione

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Jane Massey Licata

Typed Name: **Jane Massey Licata**, Reg. No. **32,257**

Commissioner for Patents
Mail Stop AF
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

APPEAL BRIEF

Table of Contents

Appendix 1 - Pending Claims

Appendix 2 - Proposed Amendment to Claim 3

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The real party of interest is Rutgers, The State University of New Jersey, assignee of all rights, title and interest in the instant application.

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VIII. Arguments

A. Rejection of Claims Under 35 U.S.C. 103(a)

There are four pending rejections under 35 U.S.C. 103(a) and each rejection relies on the same primary reference. The four separate rejections then involve four different combinations of secondary references.

The Examiner has rejected claims 1, 2-6 and 11-14 under 35 U.S.C. § 103(a) as being unpatentable over Wallace et al. (1996), in view of Holm et al. (1995), and further in view of Farber et al. (1992). The Examiner suggests that Wallace et al. (1996) disclose

methods for identification of domains within proteins while Holm et al. (1995) disclose determination of 3D structure by crystallography or NMR followed by database analysis using the DALI method. The Examiner suggests that Holm et al. (1995) disclose a comparison between urease and adenosine deaminase in which the complete 3D structure of the 352 amino acid adenosine deaminase protein is compared to the larger urease and where a comparison was performed for Adenovirus type 5 knob domain of 195 amino acids. The Examiner acknowledges that the combination of these two references does not teach or suggest the step of parsing a target polynucleotide into at least one putative polypeptide domain. However, the Examiner suggests that Farber et al. (1992) discloses a method for discriminating open reading frames in DNA, and that it would have been obvious for one of ordinary skill to combine these three references in order to maximize the useable databases to identify homologous proteins and thereby determine function of unknown proteins.

Although the Examiner was silent as to the status of this rejection in the Advisory Action dated March 3, 2003, this rejection was maintained in the last Office Action dated September 16, 2002. The Examiner rejected claims 1, 5-9 and 11-14 under 35 U.S.C. § 103(a) as being unpatentable over Wallace et al. (1996), in view of Holm et al. (1995), and further in view of Friedrichs et al. (1994). The Examiner suggests that Wallace et al. (1996)

disclose methods for identification of domains within proteins while Holm et al. (1995) disclose determination of 3D structure by crystallography or NMR followed by database analysis using the DALI method. The Examiner suggests that Holm et al. (1995) disclose a comparison between urease and adenosine deaminase in which the complete 3D structure of the 352 amino acid adenosine deaminase protein is compared to the larger urease and where a comparison was performed for Adenovirus type 5 knob domain of 195 amino acids. The Examiner acknowledges that the combination of these two references does not teach or suggest the step of parsing a target polynucleotide into at least one putative polypeptide domain, nor the use of NMR characterization techniques. However, the Examiner suggests that Friedrichs et al. (1994) teaches determination of the correctness of protein structure using a variety of NMR spectrometry spectra and automated analysis of these spectra using a computer program as well as amide hydrogen exchanges.

Again, although the Examiner was silent as to the status of this rejection in the Advisory Action dated March 3, 2003, this rejection was maintained in the last Office Action dated September 16, 2002. The Examiner rejected claims 1 and 5-14 under 35 U.S.C. § 103(a) as being unpatentable over Wallace et al. (1996), in view of Holm et al. (1995), and further in view of Friedrichs et al. (1994) and Bagby et al. (1997). The Examiner suggests that Wallace et al. (1996) disclose methods for identification of

domains within proteins while Holm et al. (1995) disclose determination of 3D structure by crystallography or NMR followed by database analysis using the DALI method. The Examiner suggests that Holm et al. (1995) disclose a comparison between urease and adenosine deaminase in which the complete 3D structure of the 352 amino acid adenosine deaminase protein is compared to the larger urease and where a comparison was performed for Adenovirus type 5 knob domain of 195 amino acids. The Examiner acknowledges that the combination of these two references does not teach or suggest the step of parsing a target polynucleotide into at least one putative polypeptide domain, nor the use of NMR characterization techniques, nor the button test for microdialysis and NMR. However, the Examiner suggests that Friedrichs et al. (1994) teaches determination of the correctness of protein structure using a variety of NMR spectrometry spectra and automated analysis of these spectra using a computer program as well as amide hydrogen exchanges, while Bagby et al. (1997) teach a method for preparing samples for NMR to determine optimal solubilization using a button test.

Finally, with respect the fourth remaining rejection under 35 U.S.C. 103(a), although the Examiner was silent as to the status of this rejection in the Advisory Action dated March 3, 2003, this rejection was maintained in the last Office Action dated September 16, 2002. The Examiner has rejected claims 1-9 and 11-14 under 35

U.S.C. § 103(a) as being unpatentable over Wallace et al. (1996), in view of Holm et al. (1995), and further in view of Farber et al. (1992) and Friedrichs et al. (1994). The Examiner suggests that Wallace et al. (1996) disclose methods for identification of domains within proteins while Holm et al. (1995) disclose determination of 3D structure by crystallography or NMR followed by database analysis using the DALI method. The Examiner suggests that Holm et al. (1995) disclose a comparison between urease and adenosine deaminase in which the complete 3D structure of the 352 amino acid adenosine deaminase protein is compared to the larger urease and where a comparison was performed for Adenovirus type 5 knob domain of 195 amino acids. The Examiner acknowledges that the combination of these two references does not teach or suggest the step of parsing a target polynucleotide into at least one putative polypeptide domain. However, the Examiner suggests that Farber et al. (1992) discloses a method for discriminating open reading frames in DNA, and that it would have been obvious for one of ordinary skill to combine these three references in order to maximize the useable databases to identify homologous proteins and thereby determine function of unknown proteins. The Examiner suggests that Friedrichs et al. (1994) teaches the use of NMR techniques.

Appellant respectfully disagrees with the Examiner's suggestions regarding each of the four rejections under 35 U.S.C. 103(a) that are on appeal.

1. Summary of the Teachings of Each Reference Cited Under 35 U.S.C. 103(a)

Wallace et al. (1996) teach derivation of 3-dimensional coordinate templates that have been derived from known 3-dimensional protein structures which are provided in a database. The paper then teaches determination of biochemical function based on the existence of the known 3-dimensional structures. The critical step in the method is the identification of a triad of amino acids, Ser-His-Asp, that occur in a 3-dimensional configuration to form an active site of a domain. No other size of a putative domain is taught or suggested by this reference. In fact, it is this 3 amino acid sequence that is then used to guide identification of the functions of the unknown proteins as the authors report that this triad is what is critical for differentiating catalytic from non-catalytic proteins among the serine proteases and lipases. As a result, this paper would teach away from any suggestion that a domain of 50 to 300 amino acids, such as claimed in the instant invention, would be useful for comparison to protein data bases and then identifying

proteins. Further, as the authors state at page 1002, last paragraph in the first column, their method had specific steps that involved using a data set of serine proteases and lipases to automatically compute a highly specific 3D template for the Ser-His-Asp catalytic triad in the set of proteins. As they also state at page 1002, top of the second column, their method differed from other previous methods in that a simple template specific to the Ser-His-Asp catalytic triad is derived. Then, the next step was to search for similar triads in other proteins to see how often they occurred outside of the serine proteases and lipases. Thus, the teaching of this paper is limited to teaching a skilled artisan that databases may be searched using a specific 3D template, the triad, and then used to identify a potential biological function of an unknown protein. This paper also fails to teach either the prestep of the instant claims that involves parsing a database to identify a protein coding region, as well as failing to provide any motivation to predict exon boundaries in a nucleotide sequence to identify protein domains.

Holm et al. (1995) is a commentary article wherein the DALI method is disclosed as being useful for the study of protein structure. In this method, structural relatedness is measured in terms of similarities of intramolecular distance matrices. The importance of this new automated method is

discussed. Although the Examiner suggests that this paper teaches a comparison that was performed on the Adenovirus type 5 knob domain which is a 195 amino acid comparison, nowhere does this paper teach or suggest that there was specifically such a size comparison. In fact, the table referred to by the Examiner (table 1) contains no such useful size information. Accordingly, contrary to the Examiner's suggestion, this paper does not teach a method as claimed which recites identifying a putative polypeptide domain consisting of 50 to 300 amino acids. This paper also fails to teach a method which has any specific steps at all, including a prestep of parsing a database to identify a protein domain. It also does not suggest performing a structural analysis of a protein of unknown function.

Farber et al. (1992) disclose a neural network and information theory for determination of coding regions of DNA sequence. This paper, from a different art area (not protein biochemistry as is the are of one of skill in the instant invention), fails to teach or suggest a method such as the claimed invention. Nowhere is there any teaching or suggestion of the identification of protein or polypeptide domains of 50 to 300 amino acids. Moreover, being in a different art area, this paper would not be one that would be

used by one of skill in protein biochemistry as motivation to produce the method of the instant invention.

Friedrichs et al. (1992) is a review type paper that teaches an automated NMR procedure. This paper fails to teach or suggest a method with specific steps such as is claimed in the instant invention. In particular, there is no teaching of first parsing a target polypeptide that encodes a protein of unknown function into putative polypeptide domains, followed by the identification of protein or polypeptide domains of 50 to 300 amino acids, and then, after use of NMR techniques for protein structure determination, correlating structure with biochemical functions.

Bagby et al. (1997) teach methods for preparing samples for NMR analysis, specifically a button test method. This method is used by the authors to screen solution conditions to determine conditions under which a protein is soluble at high concentrations that are used for NMR spectroscopy. This paper fails to teach or suggest a method with specific steps such as is claimed in the instant invention. In particular, there is no teaching of first parsing a target polypeptide that encodes a protein of unknown function into putative polypeptide domains, followed by the identification of protein or polypeptide domains of 50 to 300 amino acids, and then, after

use of NMR techniques for protein structure determination, correlating structure with biochemical functions.

Therefore, Appellants respectfully disagree with the Examiner's suggestion that the cited combination of prior art references establishes a *prima case* of obviousness.

2. If an Independent Claim is Nonobvious Then a Claim Depending Therefrom is Nonobvious (MPEP 2143.03)

At the outset, Appellants will address the four rejections under 35 U.S.C. as a group since in each case the only independent claim, claim 1, has been included in the rejections. Appellants respectfully point out that under MPEP 2143.03, "If an independent claim is nonobvious under 35 U.S.C. 103, then any claim depending therefrom is nonobvious (In re Fine, 837 F.2d 1071, 5 USPQ2d 1596, Fed. Cir. 1988)."

3. Three Basic Criteria of *prima facie* Obviousness

In accordance with MPEP § 2143, to establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the reference itself or in the knowledge generally available to one of ordinary skill in the art, to modify the reference. Second, there must be a reasonable expectation of success. Finally, the prior art must teach or suggest all of the claim

limitations. The prior art combinations cited under 35 U.S.C. 103 by the Examiner fail to teach or suggest all of the limitations of the claims, teach toward other limitations, and fail to provide either the motivation to modify the reference or the expectation of success.

4. The Cited Art Fails to Teach the Limitations of the Claims

None of the suggested combinations of prior art teach or suggest two of the limitations in the claims, namely the use of putative polypeptide domains of 50 to 300 amino acids and the pre-step of parsing the target polypeptide, one that encodes a protein of unknown function, into at least one putative polypeptide domain. The primary reference cited by the Examiner, Wallace et al. (1996), fails to teach or suggest either of these limitations as discussed above. In fact, Wallace et al. (1996) teaches the critical nature of a triad. In considering the secondary references cited, the fact that one comparison in the paper by Holm et al. (1995) happened to be 195 amino acids is not the same as teaching or suggesting that size is an important factor as is claimed in the instant invention. Both the MPEP and case law are quite clear. To establish *prima facie* obviousness of a claimed invention, all the limitations must be taught or suggested by the prior art.

In re Royka, 490 F.2d 981, 180 USPQ 580 (CCPA 1974) and MPEP 2143.03. Since none of the prior art references, either alone or when combined, teach or suggest the limitations of first parsing a target polypeptide, one that encodes a protein of unknown function, into at least one putative polypeptide domain and then identifying a putative polypeptide domain that properly folds into a stable polypeptide domain of 50 to 300 amino acids, the cited combination of art cannot render the instant invention obvious as set forth in claim 1 or dependent claims therefrom because there is no explicit teaching of these limitations.

5. The Combined References Fail to Provide Motivation

The cited combinations of references also fail to establish a *prima facie* case of obviousness because the motivation to combine these references is lacking. The teaching or suggestion must be found in the prior art and not based on applicant's disclosure. *In re Vacek*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). The mere fact that the cited references can be combined or modified does not render the resultant combination obvious unless the prior art suggests the desirability of the combination. MPEP 2143.01. *In re Mills*, 916 F.2d 680, 16 USPQ2d 1430 (Fed. Cir. 1990). It is only the hindsight vision afforded by the claimed invention

could provide motivation to consider Wallace et al., Holm et al., and Farber et al. Wallace et al. and Holm et al. are protein biochemistry articles directed at the analysis of protein structure and function. Farber et al. is from the art area of information theory. Contrary to the Examiner's suggestion, it would not have been obvious to the ordinary protein biochemist after reading these disclosures to take the additional step of predicting exon boundaries in a polynucleotide sequence to identify protein domains.

Further, since the reference of Wallace et al. teaches the importance of their triad domain, one of skill in the art would not be motivated to look for some other size limitation to apply to a method such as the claimed method.

Therefore, solely on the basis of the requirements for establishing a *prima facie* case of obviousness (MPEP 2143), any of the combinations of the cited references fail to make claim 1, and by dependency claims 3-14, obvious.

6. The Cited References Fail to Provide a Reasonable Expectation of Success

The primary reference cited in each of the four remaining 103 rejections, Wallace et al. (1996), is also important in that it would actually teach away from an expectation of success for the present method. As discussed in detail *supra*,

the method of Wallace et al. is based on the importance of the identification of the triad domain. It is only with use of this domain as a screening tool that the authors report any success at correlating structure of a protein with a function. The present method, however, is not based on this triad, and in fact is based on a very different type of domain. Accordingly, this primary reference would lead one of skill to not expect success using the current method which is not limited to use of the triad disclosed by Wallace et al.

Therefore, solely on the basis of the requirements for establishing a *prima facie* case of obviousness (MPEP 2143), these combined references, in any cited combination, fail to make claims 1 and 3-14 obvious.

IX. Conclusion

The references cited by the Examiner clearly do not provide the requisite teaching or suggestion to render the claimed

invention obvious. Accordingly, it is respectfully requested that the rejections under 35 U.S.C. be withdrawn.

Respectfully submitted,

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Appendix 1 - Pending Claims

Claim 1: A high-throughput method for determining the biochemical function of a protein or polypeptide domain of unknown three dimensional structure and function comprising:

(A) parsing a target polynucleotide into at least one putative polypeptide domain;

(B) identifying a putative polypeptide domain consisting of 50 to 300 amino acids that properly folds into a stable polypeptide domain consisting of 50 to 300 amino acids;

(C) determining three dimensional structure of the stable polypeptide domain;

(D) comparing the determined three dimensional structure of the stable polypeptide domain to known three-dimensional structures in a protein data bank, wherein said comparison identifies known structures within said protein data bank that are homologous to the determined three dimensional structure; and

(E) correlating a biochemical function corresponding to the identified homologous structure to a biochemical function for the stable polypeptide domain.

Claim 3: The method according to claim 2, wherein said parsing is performed by a first computer algorithm, wherein said first computer algorithm is selected from the group consisting of a computer algorithm capable of determining exon phase boundaries of a polynucleotide, and a computer algorithm capable of determining interdomain boundaries encoded in a polynucleotide.

Claim 4: The method of claim 3, further comprising a computer algorithm that compares the putative polypeptide domain sequence with known domain sequences stored within a database.

Claim 5: the method of claim 1, wherein said identification of the stable polypeptide domain having a defined three dimensional structure is performed by a set of activity-independent biophysical criteria that assesses the correctness of folding of the polypeptide domain said set of activity-independent biophysical criteria including at least one of the criteria selected from the group consisting of circular dichroism measurements, ^{1}H-NMR spectroscopy, amide hydrogen-deuterium time course exchange, and thermal denaturation.

Claim 6: The method of claim 1, wherein said determination of the three dimensional structure of the stable polypeptide domain is obtained from an NMR spectrometer spectra of said polypeptide domain.

Claim 7: The method of claim 6, wherein said NMR spectrometer spectra include one or more spectra selected from the group consisting of nuclear Overhauser effect spectroscopy (NOESY), pulsed-field gradient ^{15}N-heteronuclear single-quantum coherence spectroscopy (PFG-HSQC), pulsed-field gradient triple-resonance HCCNH^{13}C-^{13}C total correlation spectroscopy (PFG-HCCNH-TOCSY), pulsedfield gradient HCC(CO)NH^{13}C-^{13}CTOCSY (PFG-HCC(CO)NH-TOCSY), HCCH COSY, HCCNH-TOCSY, HNCO, CANH, CA(CO)NH, CBCNH, CBCA(CO)NH, H(CA)NH, and H(CA)(CO)NH.

Claim 8: The method of claim 6, wherein said NMR spectra is analyzed by a second computer algorithm that automatically assigns resonance assignments to the polypeptide sequence.

Claim 9: The method of claim 1, wherein said identification of said stable polypeptide domain comprises measuring a time course of amide hydrogen-deuterium exchange.

Claim 10: The method of claim 1, wherein prior to step (B), said polypeptide domain is optimally solubilized, said optimum solubilization comprising:

I) preparing an array of microdialysis buttons, wherein each of said microdialysis buttons contains at least 1 µl of an approximately 1 M solution of said stable polypeptide domain;

ii) dialyzing each member of said array of microdialysis buttons against a different dialysis buffer;

iii) analyzing each of said dialyzed microdialysis buttons to determine whether said stable polypeptide domain has remained soluble; and

iv) selecting the polypeptide domain having optimum solubility characteristics for NMR spectroscopy.

Claim 11: The method of claim 1, wherein said comparison of said determined three dimensional structure to said known three-dimensional structures in the protein data bank is performed by a third computer algorithm that is capable of determining 3D structure homology between said determined three dimensional structure and a member of said protein data bank.

Claim 12: The method according to claim 11, wherein said third computer algorithm is selected from the group consisting of DALI, CATH, AND VAST.

Claim 13: The method of claim 1, wherein said protein data bank is Protein Data Base ("PDB").

Claim 14: The method of claim 4, wherein said database contains domain sequence information of known and determined domain sequences.

Appendix 2: Proposed Amendment to Claim 3

Claim 3: The method according to ~~claim 2~~ claim 1, wherein said parsing is performed by a first computer algorithm, wherein said first computer algorithm is selected from the group consisting of a computer algorithm capable of determining exon phase boundaries of a polynucleotide, and a computer algorithm capable of determining interdomain boundaries encoded in a polynucleotide.